ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2009, p. 1468–1475 Vol. 53, No. 4
0066-4804/09/$08.00+0 doi:10.1128/AAC.00339-08

Pharmacokinetics of Hydroxychloroquine and Its Clinical Implications in Chemoprophylaxis against Malaria Caused by *Plasmodium vivax*▽

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Received 11 March 2008/Returned for modification 2 July 2008/Accepted 17 January 2009

Hydroxychloroquine (HCQ) is an antimalarial drug used as chemoprophylaxis against malaria caused by *Plasmodium vivax* in the Republic of Korea Army (ROKA). In this study, we evaluated the pharmacokinetics (PK) of HCQ and its metabolites and the relationship between the PK of HCQ and the effect of treatment of HCQ on vivax malaria in South Koreans. Three PK studies of HCQ were conducted with 91 healthy subjects and patients with vivax malaria. Plasma concentrations were analyzed by noncompartmental and mixed-effect modeling approaches. A two-compartment model with first-order absorption best described the data. The clearance and the central and peripheral volumes of distribution were 15.5 liters/h, 733 liters, and 1,630 liters, respectively. We measured the plasma concentrations of HCQ in patients with prophylactic failure of HCQ and compared them with the prediction intervals of the simulated concentrations for HCQ from the final PK model built in this study. In 71% of the patients with prophylactic failure, the plasma concentrations of HCQ were below the lower bounds of the 95% prediction interval, while only 8% of them showed higher levels than the upper bounds of the 95% prediction interval. We report that a significant cause of prophylactic failure among the individuals in ROKA was ascribed to plasma concentrations of HCQ lower than those predicted by the PK model. However, prophylactic failure despite sufficient plasma concentrations of HCQ was confirmed in several individuals, warranting continued surveillance to monitor changes in the HCQ susceptibility of *Plasmodium vivax* in the Republic of Korea.

Malaria is the most prevalent parasitic disease in the world, with an estimated 500 million cases arising annually and with 1 million to 3 million deaths being attributed to this disease (20). Furthermore, most victims of malaria are below 7 years of age. Of the four species of *Plasmodium* that can cause human malaria, *Plasmodium vivax*, the causative agent of vivax malaria, is the second most common species of malaria, with an estimated 35 million *P. vivax*-transmitted malaria cases occurring worldwide each year (8).

Chloroquine (CQ), a 4-aminoquinoline compound, has been used for the prophylaxis and treatment of malaria. It acts on the ring forms of the parasites, which are relatively resistant to the action of quinine (23). CQ is known to exert its effect directly on the parasite's heme polymerization process and/or indirectly on the parasite's hemoglobin digestive pathway (2, 21). CQ was most commonly used during the 1950s to the 1960s, but its efficacy has gradually decreased to the extent that it has now been rendered completely ineffective for the prevention or treatment of malaria caused by *P. falciparum* for travelers to many areas (12). Several strains of *P. vivax* resistant to CQ have also emerged in some areas (15, 18). Hydroxychloroquine (HCQ) is an analogue of CQ in which one of the *N*-ethyl substituents of CQ is β-hydroxylated. The activity of HCQ against malaria is equivalent to that of CQ, and HCQ is preferred over CQ when high doses are required because of the lower level of ocular toxicity of HCQ than of CQ (6).

Unlike other microorganisms whose antimicrobial resistance can be tested for by incubation of the microorganism in a culture medium that contains specific antibiotics, the resistance of *P. vivax* to various antimalarial agents cannot be analyzed in this manner since an optimal system for the in vitro culture of the parasite has not yet been established. Therefore, drug resistance in *P. vivax* is usually clinically diagnosed prior to final confirmation. To confirm drug resistance in *P. vivax*, the plasma drug concentration in the patient is analyzed to verify whether treatment (or prophylactic) failure is due to decreases in the drug susceptibility of the parasites. In particular, additional data are needed to confirm prophylactic resistance in large-scale chemoprophylaxis studies, in which not every subject can be closely supervised. Knowledge of the pharmacokinetic (PK) characteristics of HCQ in healthy individuals, including PK parameters and the time-concentration

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▽ Published ahead of print on 2 February 2009.

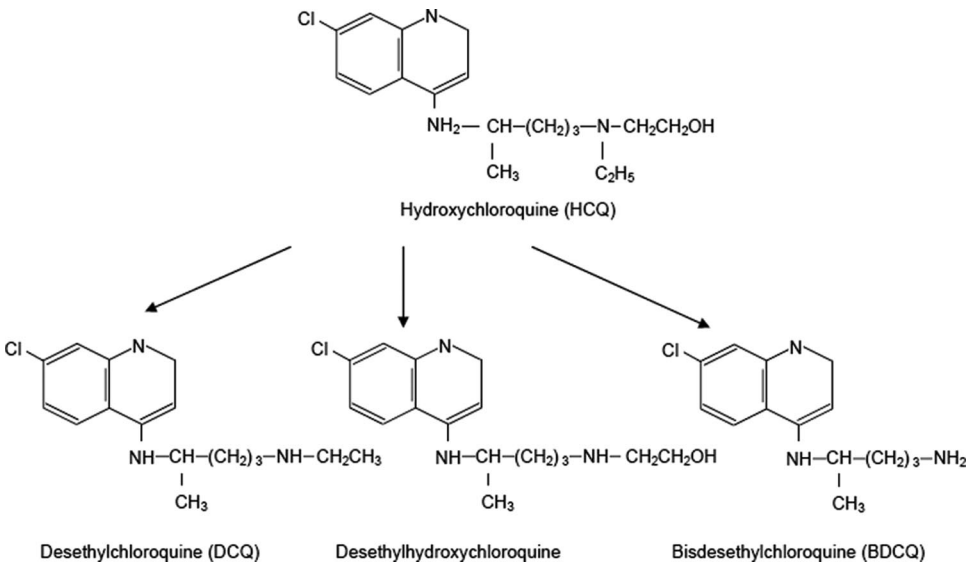

FIG. 1. Metabolism of HCQ.

profile, is required to explore the reason for prophylactic failure.

In the past several decades, various PK parameters of CQ for individuals in the Western hemisphere have been published (9, 24, 26), and studies have been performed in an attempt to compare the disposition of CQ in healthy as well as malaria parasite-infected adult subjects in Thailand (7). HCQ is almost completely and rapidly absorbed after oral administration. About 50% of the HCQ in plasma is bound to plasma proteins. HCQ is metabolized in the liver into three active metabolites: desethylchloroquine (DCQ), desethylhydroxychloroquine, and bisdesethylhydroxychloroquine (BDCQ) (Fig. 1) (13). Thus far, a study of the PK characteristics of CQ or HCQ among South Korean individuals has not been conducted. For exact confirmation of the reason for the failure of prophylaxis for vivax malaria in South Korean patients, the PK characteristics of HCQ in South Korean individuals had to be analyzed, as previous studies have shown that several drugs demonstrate differences in their population PKs by ethnicity or race (4, 22).

In this study, we evaluated the PK characteristics of HCQ and its metabolites in South Korean subjects and their relationship with the efficacy of HCQ against *P. vivax* malaria parasites among South Korean patients.

MATERIALS AND METHODS

Subject and study design. The current study consisted of two parts. The first was a PK study which aimed to obtain an adequate model of the PKs of HCQ after the oral administration of HCQ sulfate. The second was a clinical comparison study that was conducted in order to compare the real plasma concentrations from individuals who were orally medicated with HCQ sulfate to the simulated values from the PK model in these studies (Fig. 2).

In our studies, a total of 431 plasma samples were prepared from 22 healthy subjects and 69 patients with vivax malaria for three different studies (studies Ia, Ib, and II). In study Ia, a single dose of HCQ sulfate of 400 mg (310 mg as HCQ) was administered to six healthy South Korean male volunteers and serial, frequent blood samples (8 ml each) were collected at time zero (prior to drug administration) and at 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, 72, 144, and 288 h after the administration of HCQ sulfate. This provided the data for selection of the PK structural model. In study Ib, another 16 healthy adult subjects were administered an oral dose of HCQ sulfate of 400 mg; and blood samples were collected at 0, 3, 72, and 168 h after drug administration. In study II, 69 civilian patients with vivax malaria were administered HCQ sulfate at 800 mg and were then administered HCQ sulfate at 400 mg at 6, 24, and 48 h afterward. These patients had febrile illness, and all of them were diagnosed with vivax malaria from May to October 2003 by microscopic examination of peripheral blood smears stained with Giemsa. After the diagnosis was made, the patients were administered HCQ sulfate under the supervision of a physician. Blood samples were drawn from each patient at time zero (the baseline); 24, 48, and 72 h after administration of the first dose; and 6 to 9 days after administration of the last dose.

To evaluate the reasons for prophylactic failures, a clinical comparison study was conducted in which blood samples were collected from 61 soldier patients who had developed vivax malaria from 2000 to 2003, despite the administration of prophylactic doses of HCQ sulfate. The blood samples were used to measure the plasma concentrations of HCQ. The plasma concentrations of HCQ were compared to the simulated time-concentration profiles for the prophylactic medication of HCQ sulfate based on the PK model developed from the current PK studies.

In these studies, one brand of HCQ sulfate was used to treat all subjects. Blood samples were centrifuged at $250 \times g$ for 10 min at 4°C, and the plasma obtained was immediately stored in polypropylene tubes at −70°C until further analysis.

Human use protocol. All protocols for these studies were reviewed and approved by the Institutional Review Board of the Gil Medical Center (Incheon, Republic of Korea), and all the procedures were conducted in accordance with the recommendations of the Declaration of Helsinki on biomedical research involving human subjects. The subjects in studies Ia and Ib were proved to be healthy after comprehensive medical examinations, including a review of their medical histories, physical examination, determination of vital signs, 12-lead electrocardiography, and routine clinical laboratory tests within the 3 weeks before the administration of HCQ sulfate. All the subjects were within 15% of their ideal body weight and had no history of smoking or heavy drinking within 3 months of the study. None of these subjects had taken any medicine within 7 days prior to the commencement of the study. The subjects were not allowed to smoke, consume alcoholic beverages, or ingest caffeine-containing beverages and/or food during the study. They were also instructed to refrain from vigorous activities. The subjects fasted from 10 h prior to the dosing of HCQ sulfate through 4 h after the dosing. All subjects gave written informed consent before any procedures related to this study were performed. The informed consent included information on the regimen, the blood collection schedule, medical examination, the efficacy and possible side effects of the drug, retraction from participation, management of the private database of the volunteers, etc.

Measurement of plasma drug concentrations. HCQ, DCQ, and BDCQ were provided by the U.S. Centers for Disease Control and Prevention (Atlanta, GA). The internal standard, 2,3-diaminoaphthalene, was obtained from Sigma (St. Louis, MO). Plasma concentrations of HCQ and its metabolites, DCQ and

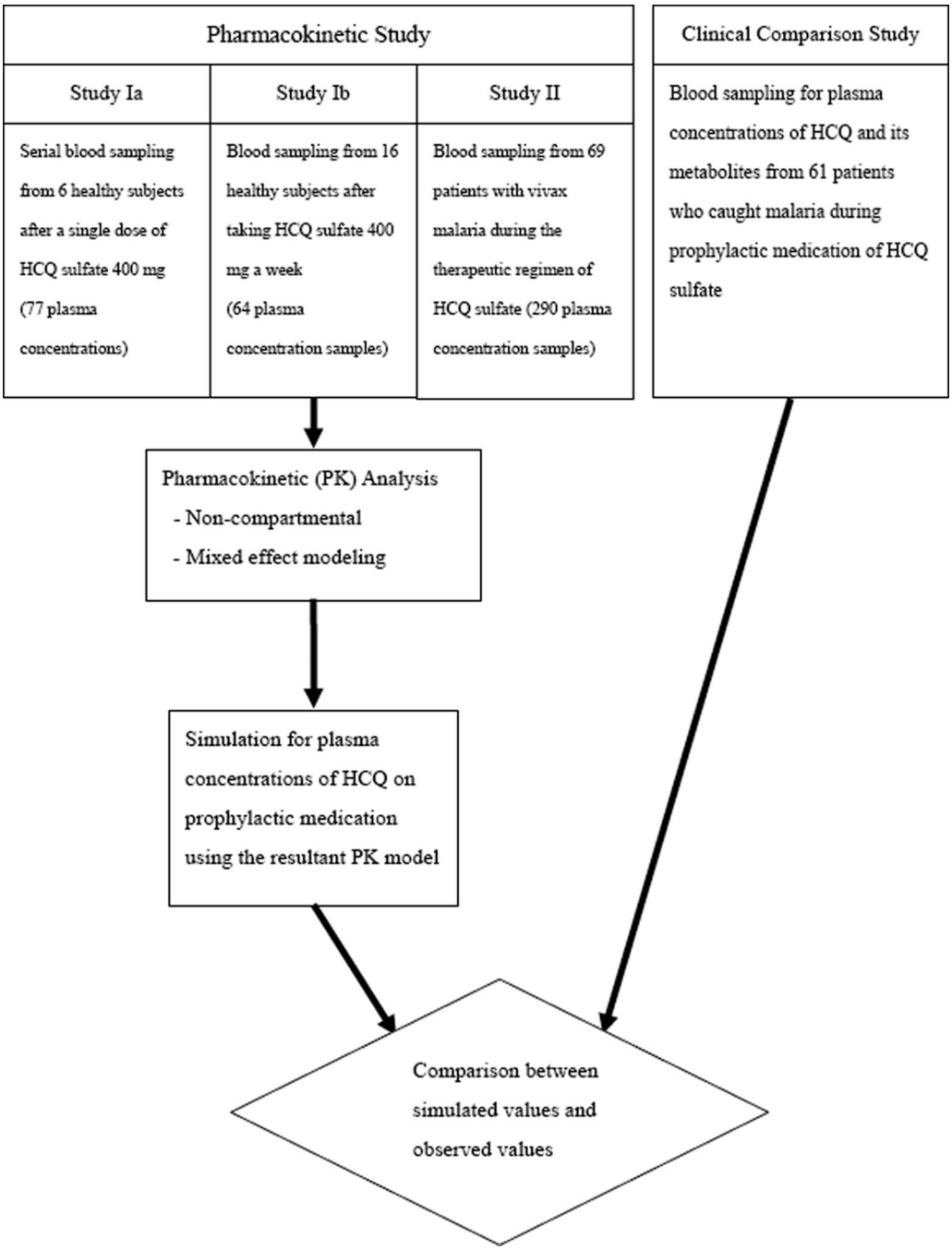

FIG. 2. Overall study flow.

BDCQ, were measured by a validated reversed-phase high-performance liquid chromatography method, as described by Easterbrook (13), with slight modifications. In brief, 0.4 ml of each plasma sample was mixed with 50 μl of a distilled water solution of the internal standard (0.1 μg/ml) and 250 μl of 1 M ammonium solution. Extraction was performed with 6 ml of diethyl ether. The supernatant obtained after centrifugation was desiccated with a Speed-Vac apparatus at −80°C. One hundred twenty microliters of the mobile phase was injected into the sample, and the mixture was vortex mixed. Then, 90 μl of the sample was injected into the high-performance liquid chromatograph. Chromatography was performed on a Capcell Pak C_{18} column (particle size, 5 μm; 4.6 by 150 mm) at room temperature at a flow rate of 1.0 ml/min. The compounds were quantified with a fluorescence detector set at an excitation wavelength of 320 nm and an emission wavelength of 370 nm. The mobile phase consisted of acetonitrile and 0.02 M phosphate buffer (389:1,000, vol/vol; pH 4.9). The method was validated in the range of 10 to 2,000 ng/ml (10, 20, 50, 100, 200, 500, 1,000, 2,000 ng/ml) for HCQ and 5 to 200 ng/ml (5, 10, 20, 50, 100, 200 ng/ml) for DCQ and BDCQ. Intra- and interassay coefficients of variation varied from 3.1% to 5.2% and from 3.5 to 7.3%, respectively, for HCQ at 10, 50, 1,000, and 2,000 ng/ml; from 7.8% to 12.2% and from 8.1 to 11.8%, respectively, for DCQ at 5, 20, 100, and 200 ng/ml; and from 7.2% to 12.5% and from 7.8 to 12.7%, respectively, for BDCQ at 5, 20, 100, and 200 ng/ml. The intra- and interassay accuracies were less than 15% for all the compounds. The lower limits of quantification were 10 ng/ml and 5 ng/ml for DCQ and BDCQ, respectively, and the intra- and interday coefficients of variation were less than 20% for all compounds. Concentrations below the lower limit of quantification prior to HCQ administration were considered to be 0 ng/ml.

TABLE 1. Demographic characteristics of 91 healthy subjects as well as subjects with malaria in the PK studies (studies Ia, Ib, and II)

Subject demographic characteristic	Study Ia (healthy males; $n = 6$)	Study Ib (healthy individuals)			Study II (vivax malaria patients)		
		Male ($n = 15$)	Female ($n = 1$)	Both genders	Male ($n = 49$)	Female ($n = 20$)	Both genders
Mean age (yr) ± SD[a]	25.6 ± 5.8	20.9 ± 1.1	20	20.8 ± 1.1	33.2 ± 12.9	45.2 ± 13.7	36.3 ± 13.6
Mean body weight (kg) ± SD	76.2 ± 10.0	67.1 ± 9.9	48	65.9 ± 10.7	69.7 ± 11.6	57.4 ± 11.4	66.4 ± 12.6
Mean ht (cm) ± SD	175.0 ± 2.8	175.0 ± 5.3	165	174.4 ± 5.7	172.2 ± 5.2	156.9 ± 5.9	168.1 ± 8.8

[a] SD, standard deviation.

PK analysis. (i) Noncompartmental analysis. Serial plasma concentration data for HCQ, DCQ, and BDCQ from six healthy subjects were analyzed by noncompartmental methods with the WinNonlin (version 5.2) program (Pharsight Corporation, Mountain View, CA). The numbers of data used in the analysis, excluding the concentrations below the limit of quantification, were 77, 59, and 66 for HCQ, DCQ, and BDCQ, respectively. The maximum drug concentrations in plasma (C_{max}) and the time to C_{max} were determined directly from the observed values. The terminal elimination rate constant (λ_z) was estimated by linear regression of the log-linear decline of at least three individual plasma time-concentration data. The terminal half-life ($t_{1/2}$) was calculated for each individual as follows: $t_{1/2} = \ln(2)/\lambda_z$. The area under the concentration-time curve (AUC) from time zero to the last measurable time (AUC_{last}) was calculated by the linear-log linear trapezoidal method. The AUC from time zero extrapolated to infinity (AUC_{inf}) was also calculated by using a combination of the linear-log linear trapezoidal method and extrapolation to infinity by using λ_z and the last observed concentration.

(ii) Analysis by mixed-effect modeling. Plasma concentration data for HCQ from all 91 subjects were analyzed by mixed-effect modeling by using the NONMEM (version VI) program (GloboMax Limited Liability Company, Hanover, MD). The PK parameters were estimated with NONMEM subroutines ADVAN4 and TRANS4 by use of the FOCE (first-order conditional estimation) with INTERACTION method. The parameters for a specific subject are described by equation 1:

$$P_i = P_{TV} \times \exp(\eta_i) \quad (1)$$

where P_{TV} is the typical value of a parameter, and η_i is a normally distributed variable with zero mean.

The residual error model was characterized by use of the combined-error mode, as described by equation 2:

$$C_{obs} = C_{pred} + (C_{pred}\varepsilon_1) + \varepsilon_2 \quad (2)$$

where C_{obs} is the observed concentration, C_{pred} is the predicted concentration, and ε_1 and ε_2 are zero mean normally distributed variables.

Various compartmental models and error models were assessed, guided by a graphical assessment of the optimum fit properties and statistical significance criteria. The covariates tested for HCQ PKs were age, sex, body weight, height, and disease status (healthy or malarial). To identify a potentially significant covariate, random permutation tests were conducted over 1,000 times for each variable or combination of variables. A likelihood ratio test was used to discriminate between the hierarchical models at a P value of ≤ 0.05, based on the fact that the distribution of the -2 log likelihood of the models approximately follows a chi-square distribution. Standard diagnostic plots, including the observed values of the dependent variable versus the individual predicted values and the individual predicted values versus the individual weighted residuals, were used for the detection of optimum fit capabilities. Other diagnostics were the objective function value and the standard error of the parameter estimates. To evaluate the stability of the model and to confirm the result, bootstrapping with wings was conducted with the NONMEM program (27). A total of 2,000 bootstrap runs were performed, and from the resultant parameter distributions, the 95% confidence intervals of the parameter estimates were obtained as 2.5th and 97.5th percentiles. The modeling process was facilitated by use of the Asan software tool for NONMEM, which is an interface for NONMEM based on text editor and the R program (19).

HCQ time-concentration profiles at the dosage used for the prophylaxis of malaria (HCQ sulfate at 400 mg a week) were simulated by using the NONMEM (version VI) program and the fixed- and random-effect parameter estimates. The 95% prediction intervals from the simulation were compared to the actual plasma HCQ concentration data that were obtained from vivax malaria patients who developed the disease, despite the previous prophylactic administration of HCQ sulfate.

RESULTS

Subjects. PK studies were conducted with 91 subjects in three different substudies (studies Ia, Ib, and II). Study Ia consisted of 6 male healthy individuals, and study Ib consisted of 15 healthy male and 1 healthy female individuals. Study II consisted of 49 male and 20 female patients with vivax malaria. The demographic characteristics of the subjects from each substudy are shown in Table 1.

PK analysis. In the noncompartmental analysis with six healthy subjects (study Ia), AUC_{inf} and C_{max} were 102.3 ± 60.8 nmol · h/ml (mean ± standard deviation) and 1.22 ± 0.40 nmol/ml, respectively, for HCQ; 37.7 ± 16.9 nmol · h/ml and 0.06 ± 0.03 nmol/ml, respectively, for DCQ; and 53.6 ± 44.5 nmol · h/ml and 0.36 ± 0.64 nmol/ml, respectively, for BDCQ (Fig. 3; Table 2). The measure of clearance divided by the measure of bioavailability (CL/F) and the volume of distribution based on the terminal elimination phase divided by the bioavailability of HCQ were calculated to be 12.0 ± 6.8 liters/h and 2,851 ± 2,147 liters, respectively.

The population PK analysis of HCQ was conducted with 431 plasma concentration data from all the 91 subjects in the PK studies by using the NONMEM (version VI) program. The model that best described the typical time course of the plasma

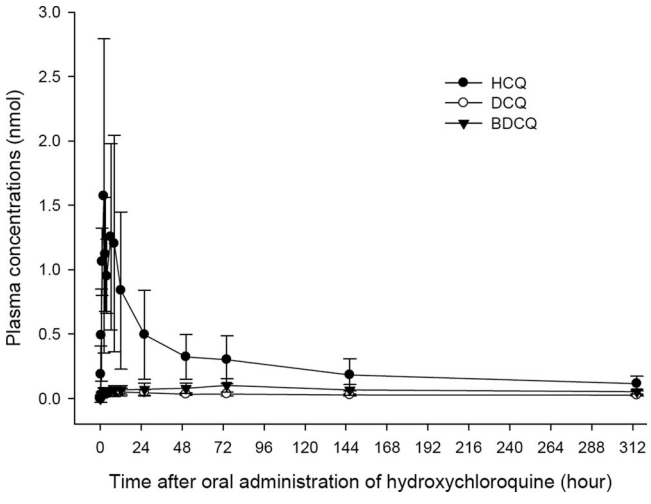

FIG. 3. Plasma concentrations (mean and standard deviation) of HCQ and its metabolites in the six healthy subjects in study Ia before and after the administration of a single oral dose of HCQ sulfate at 400 mg.

TABLE 2. Noncompartmental PK results for HCQ and its metabolites DCQ, and BDCQ, after administration of a single oral dose of HCQ sulfate at 400 mg to six healthy subjects in study Ia[a]

Drug	T_{max}[a] (h)	C_{max} (nmol/ml)	$t_{1/2}$ (h)	AUC_{last} (nmol · h/ml)	AUC_{inf} (nmol · h/ml)	CL/*F* (liters/h)	V_z/F (liters)	AUC_{DCQ}/AUC_{HCQ}[b]	AUC_{BDCQ}/AUC_{HCQ}[b]
HCQ	2.4 (2.1–3.7)	1.22 ± 0.40	172.3 ± 39.0	75.4 ± 46.9	102.3 ± 60.8	12.0 ± 6.8	2,851 ± 2,147		
DCQ	6.1 (3.0–74.2)	0.06 ± 0.03	549.9 ± 171.5	12.2 ± 5.9	37.7 ± 16.9			0.39 ± 0.29	
BDCQ	72.8 (2.1–74.2)	0.36 ± 0.64	241.0 ± 112.2	34.0 ± 34.3	53.6 ± 44.5				0.63 ± 0.62

[a] The times to peak plasma concentration are medians (ranges); all other values are means ± standard deviations. Abbreviations: T_{max}, time to peak plasma concentration; V_z, volume of distribution based on the terminal phase; *F*, fraction of dose absorbed. The other abbreviations are defined in the text.
[b] Molar ratios are displayed.

HCQ concentrations was a two-compartment linear model with first-order absorption. The model was improved significantly by adding an absorption lag from the depot compartment to the central compartment (change in the objective function value, 32.5 [from 3,334.8 to 3,302.3]). No covariate was included in the final model, since a significant tendency on graphics between the interindividual variabilities of each fixed-effect parameter estimate and the various demographic variables was not observed, and none of the variables significantly reduced the objective function value when they were incorporated into the model. CL/*F* was estimated to be 10.9 liters/h, and from this value, an average steady-state concentration of 0.51 M (170 ng/ml) was predicted. The population PK parameter estimates of HCQ and basic diagnostic plots are presented in Table 3 and Fig. 4, respectively.

Simulation of a time-concentration profile of HCQ. By using the final PK model developed in this study, 1,000 simulations were performed for the plasma concentrations of prophylactic doses of HCQ (HCQ sulfate at 400 mg a week) with the NONMEM (version VI) program. These results were compared to the actual plasma concentration data for HCQ for 61 soldier patients with vivax malaria (clinical comparison study) who became infected, despite the previous weekly chemoprophylactic administration of 400 mg HCQ sulfate for more than 4 weeks as a preventive measure. The plasma concentrations of HCQ in 43 patients (71%) were found to be below the lower bounds of the 95% prediction interval, and those in 5 patients (8%) were found to be higher than the upper bounds of the 95% prediction interval. The plasma concentrations were within the range of the 95% prediction interval in only 13 patients (21%) (Fig. 5). On the other hand, the plasma concentrations of HCQ were below the average predicted concentrations in 48 patients (79%), whereas they were above the average predicted concentrations in 13 patients (21%).

DISCUSSION

Vivax malaria was endemic on the Korean peninsula for many centuries. During the Korean War (1950 to 1953), approximately 15% of all febrile illnesses among Republic of Korea Army (ROKA) personnel were attributed to malaria (11, 16). However, this incidence decreased steadily, and in the late 1970s, the Republic of Korea was declared malaria-free (28). It was not until 1993 that vivax malaria reemerged along the demilitarized zone in the Republic of Korea. After its reemergence, the annual incidence of vivax malaria increased rapidly, reaching 4,141 cases in 2000 (17). Although there was a decrease in the annual number of vivax malaria cases to 864 in 2004, it once again increased in 2005 and reached more than 2,000 cases by 2007 (30, 31).

In an attempt to cope with the rapidly increasing rates of malaria among various military units and to prevent the spread of malaria to civilian populations throughout the Republic of Korea, chemoprophylaxis with HCQ sulfate and primaquine phosphate (terminal prophylaxis) was initiated among military personnel assigned to areas at high risk for malaria in 1997. The chemoprophylaxis program has expanded annually and included from approximately 16,000 soldiers in 1997 to 200,000 soldiers in 2007, with the cumulative number of soldiers given chemoprophylaxis reaching more than 1.4 million by the end of 2007 (29). Despite the poor compliance with therapy in several areas, the chemoprophylaxis policy instituted by the ROKA has contributed to reductions in the incidence of malaria among soldiers and veterans. However, the prophylactic administration of HCQ has also increased the possibility of the occurrence of HCQ-resistant strains of *P. vivax*. Prophylactic failures have consistently been reported since the initiation of chemoprophylaxis within the ROKA. During the early years of chemoprophylaxis, before 2000, most prophylactic failures re-

TABLE 3. Population PK parameter estimates for HCQ after administration of a single oral dose of HCQ sulfate (400 mg) in the PK studies (studies Ia, Ib, and II)[a]

Parameter	k_a (h^{-1})	ALAG (h)	IIV of ALAG	V_c (liters)	IIV of V_c	V_p (liters)	IIV of V_p	*Q* (liters/h)	CL/*F* (liters/h)	IIV of CL/*F*	ε_1 (proportional)	ε_2 (additive)
Estimated value	1.15	0.389	0.0359	437	0.232	1,390	0.715	45.1	10.9	0.161	0.27[b]	2.77[b]
% RSE	15.7	8.8	61.1	17.8	57.8	17.8	22.3	16.6	21.5	41	7.3	37.5
95% CI	0.80–1.50	0.32–0.46	−0.09	284–589	−0.53	905–1,875	0.40–1.03	30.4–59.8	6.3–15.5	0.03–0.29	0.49–0.57	0.84–4.81
Bootstrap estimate	1.09	0.39	0.15	443	0.47	1,484	0.57	47.1	11.1	0.3	0.27	2.16
Bootstrap 95% CI	0.61–1.53	0.32–0.49	0.00–0.35	230–615	0.12–0.76	919–2,141	0.44–0.71	30.6–61.3	7.9–14.6	0.19–0.37	0.23–0.29	1.43–3.63

[a] Abbreviations: RSE, relative standard error (standard error divided by the parameter estimate); CI, confidence interval; IIV, interindividual variability; k_a, absorption rate constant; ALAG, absorption lag time; V_c, central volume of distribution; V_p, peripheral volume of distribution; *Q*, intercompartmental clearance.
[b] The values represent standard deviations.

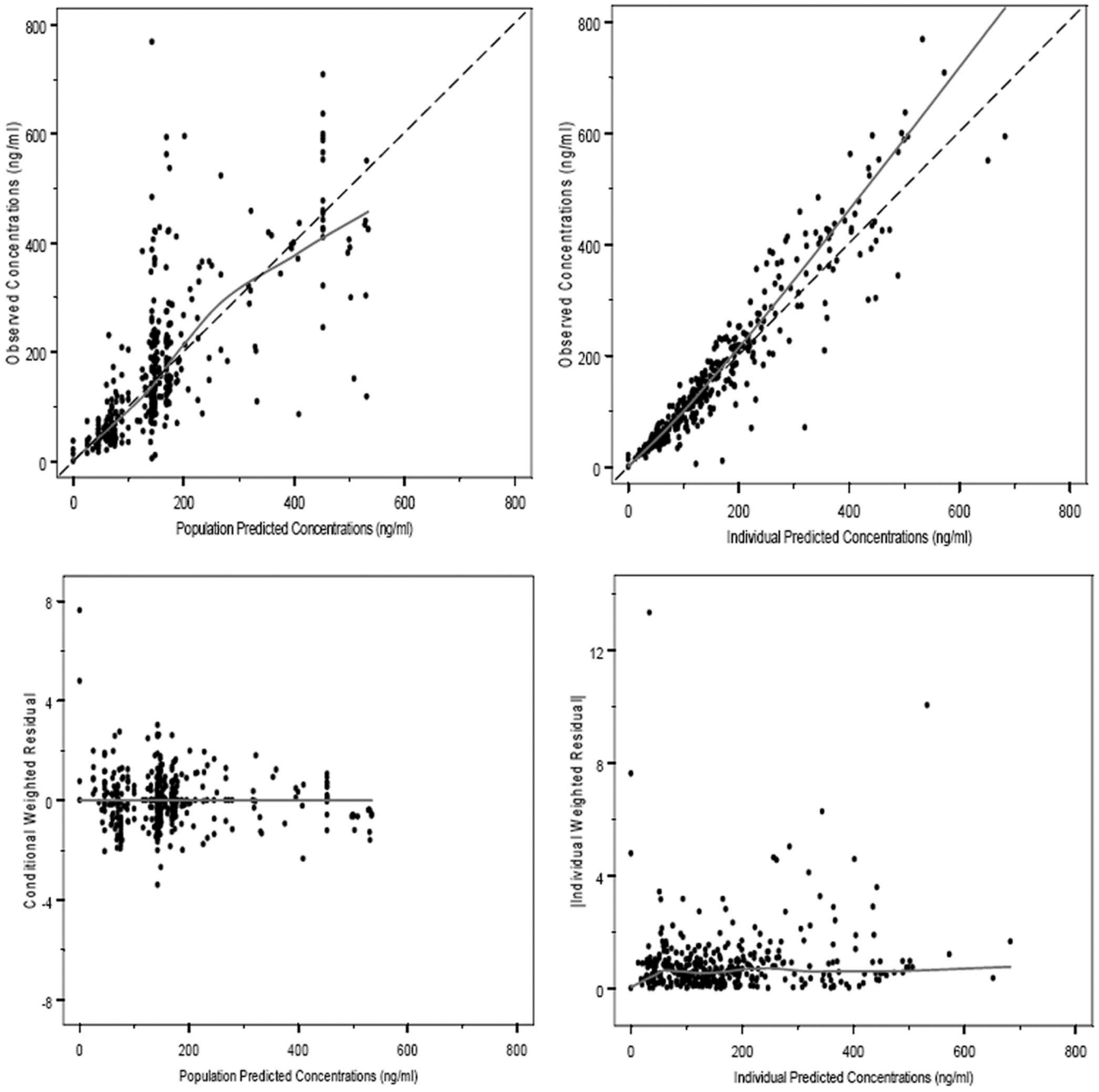

FIG. 4. Diagnostic plots for the final population PK model of HCQ in the pharmacokinetic studies (studies Ia, Ib, and II). Solid line, fit by local regression (loess); dashed line, line of identity.

sulted from poor compliance. However, since 2000, army doctors of the ROKA have consistently reported cases of prophylactic failure that developed despite the regular administration of HCQ (personal communications). Therefore, the PK characteristics of HCQ and its metabolites, such as steady-state plasma levels after chemoprophylaxis with HCQ sulfate, need to be evaluated in order to identify the reason for the failure of chemoprophylaxis and to monitor the potential HCQ resistance of *P. vivax* in the Republic of Korea.

HCQ and its metabolites are known to be enantioselective in their dispositions, and the blood concentration of the (−)-(*R*) enantiomer of HCQ was found to be higher than that of the (+)-(*S*) enantiomer of HCQ (5, 14). However, we measured the total concentrations of HCQ and its metabolites in the current studies since the antimalarial properties of the individual enantiomers of HCQ are not known, although it has been reported that the (+)-(*S*) enantiomer of CQ, which is very similar to HCQ, showed greater antimalarial activity than the (−)-(*R*) enantiomer of CQ in mice (10). Furthermore, in patients with rheumatoid arthritis, the effects of treatment with

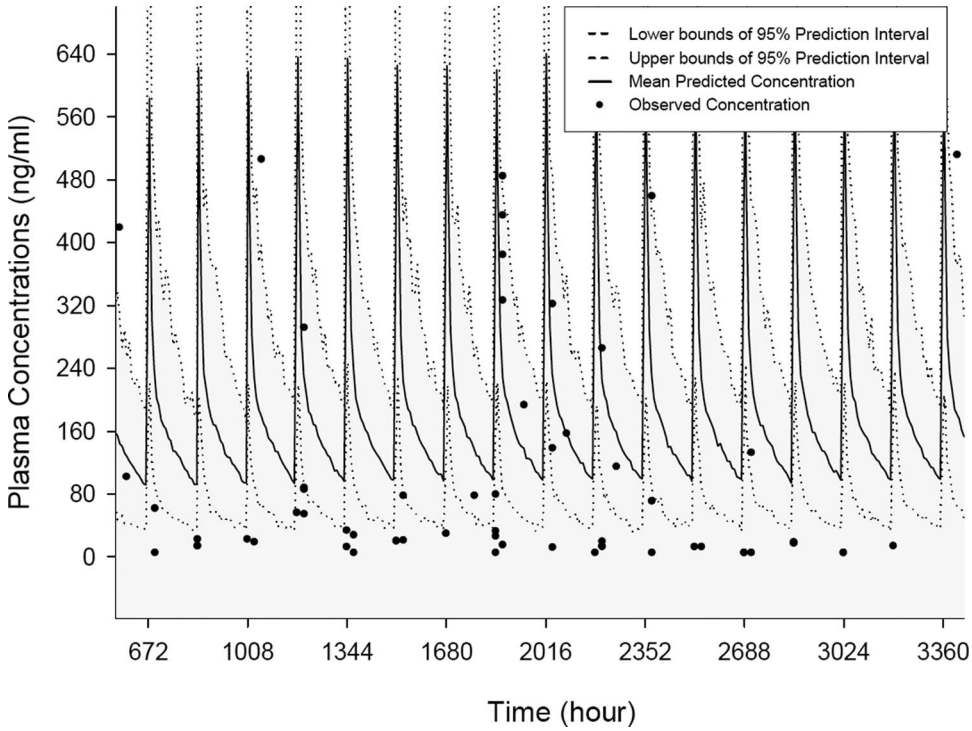

FIG. 5. Comparison of the actual plasma concentrations of HCQ in 61 soldier patients in the clinical comparison study who were infected with malaria parasites despite chemoprophylaxis for longer than 4 weeks to the simulated plasma time-concentration profiles of HCQ after oral administration of HCQ sulfate with a prophylactic dose of 400 mg/week.

HCQ have been shown to correlate with the racemic plasma concentrations (25).

Although in study Ia the plasma concentrations were measured for up to 314 h after the administration of HCQ, the AUC_{last} of each compound was found to be much smaller than the AUC_{inf}. This raises concern over the reliability of the parameter estimates. Despite this limitation, the terminal elimination phase of each compound in the concentration-time curve showed a clear log-linear decline, and we have presented the AUC_{inf} and the parameters on the basis of that value. In the mixed-effect modeling analysis, there was a tendency for the underprediction of high concentrations during the absorption phase, as can be seen in Fig. 4. We tried various absorption models, including semiphysiologic saturation models that incorporated the first-pass effect. However, in the PK analyses with various models, the parameters were not estimated precisely. Finally, the first-order absorption model with an absorption lag was chosen as the model that best described our data. From the CL/*F* estimate of 10.9 liters/h in the PK studies (studies Ia, Ib, and II), the mean steady-state plasma concentrations by use of the prophylactic regimen with HCQ was calculated to be 170 ng/ml. Therefore, considering that a CQ plus DCQ level in plasma of >10 ng/ml should achieve the complete cure of a blood-stage CQ-sensitive *P. vivax* infection, the dosage of HCQ sulfate of 400 mg once a week for the prevention of vivax malaria may be adequate (1). The PK parameters estimated in the current studies with HCQ were similar to those obtained from previous studies conducted in Australia, especially in terms of the CL/*F* (10.9 versus 9.9 liters/h), which suggests that there are no ethnic differences in the steady-state plasma concentration of HCQ after prophylactic administration (3). In the simulation, as many as 71% of 61 soldier patients in the clinical comparison study were found to have concentrations below the lower bound of the 95% prediction interval for the plasma concentrations achieved with the HCQ sulfate prophylactic regimen. This suggests that the prophylactic treatment failures were partly ascribed to their lower plasma concentrations of HCQ for various reasons, including poor compliance, malabsorption, and poor dissolution of the drug. The current PK studies (studies Ia, Ib, and II) were conducted by the use of strict controls for all procedures, including compliance with the HCQ sulfate prophylactic regimen. However, although HCQ sulfate was distributed to soldier subjects in this clinical comparison study, compliance with drug administration was not confirmed for each subject. Furthermore, except for the distribution of sex and disease status, the characteristics of the study subjects were similar between the PK studies and the clinical comparison study. However, in the model-building process, neither sex nor disease status was a significant factor that affected the PKs of HCQ. Thus, poor compliance seems be one of the main reasons for the lower plasma HCQ concentrations in patients who developed vivax malaria, despite prophylactic treatment, in the clinical comparison study.

On the other hand, of the 61 patients who developed parasitemia despite chemoprophylaxis, 5 patients (8%) showed higher concentrations of HCQ than the upper bound of the 95% prediction interval, and 13 patients (21%) showed concentrations within the range of the 95% prediction interval. In such instances, the failure of the prophylactic treatment might

be ascribed to the decreased HCQ susceptibility of the parasite, implying that certain strains of *P. vivax* in the Republic of Korea have developed resistance to the current prophylactic HCQ dosing regimen. Interestingly, these patients were successfully treated with therapeutic doses of HCQ, suggesting that the resistance to the prophylactic regimen exhibited by the particular strain of *P. vivax* had not progressed to resistance to the treatment regimen.

Many cases of vivax malaria have been reported among soldiers who had previously undergone chemoprophylaxis, raising concerns over potential drug resistance, especially if soldiers had missed scheduled treatments that would enable the parasites to develop resistance in an environment with low HCQ concentrations. A significant percentage of the prophylactic treatment failures in the ROKA was due to low plasma concentrations of HCQ, which was mainly ascribed to poor drug treatment compliance. However, resistance to prophylactic doses of HCQ was confirmed in several patients, and this warrants continued surveillance in the Republic of Korea to prevent the occurrence of *P. vivax* strains that are resistant to therapeutic doses of HCQ.

In conclusion, we evaluated the PK characteristics of HCQ and a prophylactic dose of HCQ sulfate of 400 mg once a week, which has been proposed to be adequate for the prevention of vivax malaria among individuals in the Republic of Korea, since the plasma concentrations of HCQ were high enough to exert antimalarial activity. Much of the failure of the prophylactic treatment regimen was attributed to plasma concentrations of HCQ lower than those predicted by the PK model. However, some individuals showed plasma HCQ levels within or even higher than the range of the 95% prediction interval of the model, which suggests possible resistance to the HCQ prophylactic regimen.

ACKNOWLEDGMENTS

This study was supported by a grant from the Korea Health 21 R&D project, Ministry of Health and Welfare, Republic of Korea (grant A030075), and by a grant from the Korea Centers for Disease Control and Prevention (serial number 2007-E00032-00).

We thank Mike Green of the U.S. Centers for Disease Control and Prevention for providing us with HCQ, DCQ, and BDCQ.

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